American Journal of Medical Genetics 62:179–183 (1996)

Reduction of Urinary Tract and Cardiovascular Defects by Periconceptional Multivitamin Supplementation

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The objective in the Hungarian randomised double-blind controlled trial was to study the preventive effect of periconceptional multivitamin supplementation on neural tube-defects and other congenital abnormalities. There were 2,471 and 2,391 informative offspring (prenatally diagnosed and terminated malformed fetuses, stillborn fetuses, and liveborn infants) in the multivitamin and placebo-like trace element groups, respectively. A single tablet either of a multivitamin containing 0.8 mg of folic acid or trace element supplement was given daily for at least one month before conception and at least until the date of the second missed menstrual period. The total rate of major congenital abnormalities was 20.6/1,000 in the multivitamin and 40.6/1,000 in the trace element group. After the exclusion of six cases of neural-tube defects in the trace element group the difference was very highly significant [*P* = 0.0003; relative risk of 0.54 (95% CI 0.39, 0.76)]. Multivitamin supplementation appeared to result in a significant reduction in the rate of urinary tract abnormalities, mainly obstructive defects, and in the rate of sporadic cardiovascular malformations, mainly ventricular septal defects. This report is regarded as a hypothesis-generating study encouraging others to see if the result can be repeated.

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KEY WORDS: periconceptional multivitamin supplementation, neural-tube defects, urinary tract defects, congenital cardiovascular malformations

INTRODUCTION

Periconceptional supplementation with a multivitamin, including a physiologic dose of folic acid, reduces the occurrence [Czeizel and Dudas, 1992] and recurrence [Smithells et al., 1989] of neural-tube defects (NTD), while a pharmacologic dose (4 mg) of folic acid alone reduces the recurrence of NTD [MRC Vitamin Study, 1991]. The objective of the Hungarian randomised double-blind controlled trial was to test the preventive effect of periconceptional (at least 1 month before conception and at least 6 weeks after conception) multivitamin containing 0.8 mg of folic acid supplementation on the first occurrence of NTD and other congenital abnormalities (CAs). The preliminary analysis of the Hungarian trial demonstrated a significant reduction in the total rate of other CAs but a significant difference was not found in any CA group [Czeizel, 1993]. However, the final analysis of the Hungarian trial shows a significant reduction in the rate of urinary tract and sporadic cardiovascular defects.

METHODS

The Hungarian trial as a part of the Hungarian Optimal Family Planning Programme was launched on February 1, 1984 and the randomisation of multivitamin (Composition of "multivitamin" (Elevit pronatal®): Vitamin A: 6,000 IU until the end of 1989 and 4,000 IU thereafter, B_1: 1.6 mg, B_2: 1.8 mg, nicotinamide: 19 mg, B_6: 2.6 mg, B_{12}: 4 μg, C: 100 mg, D: 500 IU, E: 15 mg, calcium pantothenate: 10 mg, biotin: 0.2 mg, folic acid: 0.8 mg, calcium: 125 mg, phosphorus: 125 mg, magnesium: 100 mg, iron: 60 mg, copper: 1 mg, manganese: 1 mg, zinc: 7.5 mg) or placebo-like trace element (Composition of "trace element": copper: 1 mg, manganese: 1 mg, zinc: 7.5 mg, vitamin C: 7.5 mg) was stopped on April 30, 1992. The analysis of pregnancy outcomes was completed on April 30, 1993 [Czeizel et al., 1994] while the data on CA including the postnatal follow-up [Czeizel and Dobó, 1994] were evaluated until the end of April, 1994.

The eligibility criteria for participation, the method of randomisation and supplement supply, the method for compliance measurement, the classification of

Received for publication June 26, 1995; revision received August 25, 1995.

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women with full, partial, and no supplementation, and the method for obtaining pregnancy outcomes were published previously [Czeizel and Dudás, 1992; Czeizel, 1993]. The evaluation was based on the intention-to-treat analysis (i.e., women were analyzed according to their random entry into the study groups independently of compliance) following the design of the MRC Vitamin Study [1991].

Informative offspring (prenatally diagnosed and terminated malformed fetuses, stillborn fetuses, and liveborn infants) were evaluated with particular attention to the occurrence and correct diagnosis or description of CAs. In Hungary all deliveries and pregnancy terminations take place in obstetrical inpatient clinics. After a report of a malformed offspring, as much clinical and pathological information as possible was obtained, including any prenatal ultrasonography films (in Hungary there is an obligatory maternal serum AFP screening combined with ultrasound study in the 16th week of gestation), a physician's detailed description (discharge summary, a written report from the examining physician, etc.), and autopsy records in all lethal cases. CAs were evaluated by an independent Data Monitoring Group every 6 months. Of course, reviewers had no prior knowledge of which informative offspring were from the multivitamin and which were from the placebo-like trace element groups. In addition, infants were examined after the 8th month of life (the average time of examination was in the 11th month) after a mail or telephone invitation to our clinic. After review of medical documents concerning previously diagnosed defects and diseases, treatments, and operations, the examination included, among other things, a pediatric evaluation performed "blindly" by two pediatricians [Czeizel and Dobó, 1994]. If families did not respond to our request, they were reinvited. If there was still no response, the infant's pediatrician was contacted and the medical history, particularly data concerning CAs diagnosed after birth, was obtained.

All informative offspring who had a CA of surgical and medical importance diagnosed before age 1 year were entered into the data-base. Thus, minor anomalies were excluded. In addition, i) positional deformities, such as torticollis (0 vs. 2), Ortolani click (40 vs. 26), different deformities of the foot (12 vs. 15), and chest (3 vs. 0), postural deformity association [Pazonyi et al., 1982] (4 vs. 6); ii) secondary microcephaly (1 vs. 1); iii) birth marks (3 vs. 3), collodion baby (1 vs. 0), and dermoid cyst (0 vs. 1); iv) possible minor anomalies, such as foramina parietale permagna (0 vs. 2) and syndactyly of toes (0 vs. 1); and v) congenital inguinal hernia (31 vs. 29) were excluded from this analysis (the numbers of infants with the above defects in the multivitamin and trace element groups are shown in parentheses). The medical diagnoses of all defects evaluated were documented appropriately and/or checked personally by our staff according to previously defined diagnostic criteria for the given CA [Czeizel et al., 1988]. The randomisation code was broken after the final diagnoses of CAs.

Two-tailed chi square and Fisher exact tests were used for statistical evaluation.

RESULTS

Of 5,502 participants with confirmed pregnancies in the final data-base, pregnancy outcomes were ascertained in 5,453 cases (dropout rate 0.9%) (Table I). The numbers of women given full, partial and no supplements were not significantly different between multivitamin and trace element groups ($\chi^2_2 = 3.96$; $P = 0.14$). Demographic factors were similar in the two groups.

The distribution of informative offspring in the intention-to-treat analysis is shown in Table II. In the follow-up study 3,962 (81.7%) infants were examined personally by the investigators and the data from 413 (8.5%) were obtained from their pediatricians, thus 4,375 infants (90.2%) were evaluated. There was no significant difference in the distribution of the above subgroups and dropouts (9.6% vs. 9.2%) between the multivitamin and trace element groups.

General Trends

CAs were differentiated on the basis of three time windows of diagnosis: i) during pregnancy (in second and third trimesters), ii) at birth, and iii) later, until and in the follow-up examination (Table III), and in addition into two main categories: isolated and multiple (≥2 different CAs in the same person) CAs [Czeizel et al., 1988]. All CA types or groups which had more than one case are shown in Table III. The rate of excluded

TABLE I. Final Data and Demographic Characteristics of the Hungarian Randomised Controlled Trial of Periconceptional Multivitamin/Trace Element Supplementation

Group	Confirmed pregnancies	Dropouts No.	Dropouts %	Evaluated pregnancies	Maternal age x	Maternal age S.D.	Proportion of primipara (%)
Multivitamin supplement							
Full course	1,980	11	0.6	1,969	27.0	3.3	88.0
Partial course	573	5	0.9	568	26.9	3.6	86.4
No supplement	266	10	3.8	256	26.1	3.2	95.3
Total	2,819	26	0.9	2,793	26.9	3.4	88.3
Trace element supplement							
Full course	1,914	5	0.3	1,909	27.0	3.3	89.4
Partial course	552	5	0.9	547	26.6	3.4	89.4
No supplement	217	13	6.0	204	26.2	3.5	96.1
Total	2,683	23	0.9	2,660	26.9	3.4	89.9

TABLE II. Distribution of Informative Offspring and Data on Infants Evaluated in the Study Groups

Informative offspring	Multivitamin group	Trace element group
Termination after prenatal diagnosis of defects	3	13[a]
Stillborn fetuses	11	9
Liveborn infants	2,457	2,369
Total	2,471	2,391
Follow-up examination of infants		
Examined (No; %)	2,002 (81.5)	1,960 (82.7)
Data obtained from pediatricians (No; %)	220 (8.9)	193 (8.1)
Evaluated (No; %)	2,222 (90.4)	2,153 (90.9)

[a]One diagnosis was false positive, another fetus had cystic fibrosis.

mild CA groups and their total rate (95 vs. 86; $\chi_1^2 = 0.21$; $P = 0.64$) did not show significant difference between the multivitamin and trace element groups.

The total rate of major CAs showed a very highly significant difference ($\chi_1^2 = 16.35$; $P < 0.0001$) between the multivitamin (20.64 per 1,000) and the trace element (40.57 per 1,000) groups; the relative risk was 0.51 (95% confidence interval 0.36, 0.71). After the exclusion of six cases with NTDs, the difference was also very highly significant ($\chi_1^2 = 13.01$; $P = 0.0003$) with a relative risk of 0.54 (0.39, 0.76).

The family history of all informative offspring was known. Of 51 offspring with CA in the multivitamin group, 3 had a similarly affected mother (one with buphthalmos had two affected daughters, while another with aortic stenosis had a daughter with atrial septal defect secundum). Of 97 offspring with defects in the trace element group, one child with cleft lip and

TABLE III. Data for Major Congenital Abnormalities (CAs) in Informative Offspring Diagnosed Prenatally (These Figures Are in Brackets), at Birth and During the Follow-Up Period in Fully Supplemented (I), Partially Supplemented (II), Unsupplemented (III), and Total Material (T) After Periconceptional Multivitamin and Trace Element Supplementation

	Multivitamin									Trace element								
	At birth (prenatal)			Follow-up			Total			At birth (prenatal)			Follow-up			Total		
CA groups	I–II	III	T	I–II	III	T	I–II	III	T	I–II	III	T	I–II	III	T	I–II	III	T
Isolated																		
Neural-tube defects	0	0	0	0	0	0	0	0	0	5 (3)	1 (1)	6 (4)	0	0	0	5	1	6
Hydrocephalus	0	0	0	0	0	0	0	0	0	1 (1)	1 (1)	2 (2)	0	0	0	1	1	2
CAs of eye (buphthalmos, coloboma)	0	0	0	2	0	2	2	0	2	0	0	0	1	0	1	1	0	1
Cardiovascular CAs	6	0	6	4	0	4	10	0	10	9	1	10	9	1	10	18	2	20
Cleft palate	0	0	0	0	0	0	0	0	0	1	1	2	0	0	0	1	1	2
Cleft lip ± palate	4	0	4	0	0	0	4	0	4	3	0	3	0	0	0	3	0	3
Pyloric stenosis	0	0	0	2	0	2	2	0	2	0	0	0	7	1	8	7	1	8
Undescended testis (confirmed after the 6th month)	0	0	0	6	0	6	6	0	6	0	0	0	10	0	10	10	0	10
Hypospadias	2	0	2	4	0	4	6	0	6	2	0	2	4	0	4	6	0	6
GAM (genital anomalies of male)	0	0	0	3	0	3	3	0	3	0	0	0	4	0	4	4	0	4
Renal agenesis	0	0	0	0	0	0	0	0	0	1 (1)	0	1 (1)	1	0	1	2	0	2
Obstructive CAs of urinary system	1 (1)	0	1	0	0	0	1	0	1	3 (2)	0	3 (2)	3	0	3	6	0	6
Reduction CAs of limb	1	0	1	0	0	0	1	0	1	5	0	5	0	0	0	5	0	5
Arthrogryposis	0	0	0	0	0	0	0	0	0	1	1	2	0	0	0	1	1	2
Other CAs	4[a]	0	4	2[b]	0	2	6	0	6	5 (1)[c]	0	5 (1)	3[d]	0	3	8	0	8
Multiple																		
Down syndrome	0	0	0	2	0	2	2	0	2	4	1	5	0	0	0	5	1	5
Ullrich-Turner syndrome	0	0	0	0	0	0	0	0	0	1 (1)	0	1 (1)	0	0	0	1	0	1
Monogenic CA-syndrome (Allagille)	1	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Unidentified multiple CA	3 (2)	1	4	3	0	3	6	1	7	3	0	3	2	1	3	5	1	6
Total	22 (3)	1	23 (3)	28	0	28	50	1	51	44 (9)	6 (2)	50 (11)	44	3	47	88	9	97

[a]Tracheal stenosis, colon malformation due to mesenterium commune, cystic kidney type II, omphalocele.
[b]Cerebellar agenesis, bronchial stenosis.
[c]Non-immune hydrops, anal atresia, exstrophy of bladder, omphalocele, postaxial polydactyly of feet.
[d]Porencephalic cyst in brain; imperforate hymen operated; severe craniosynostosis.

palate had a similarly affected father. After the exclusion of these familial cases, the difference between the two groups increased in the total rate of major CAs [$\chi_1^2 = 18.12$; $P < 0.0001$; relative risk 0.48 (0.34, 0.68)].

Specific CA Groups

NTD are related to the main hypothesis that the trial was designed to test. The difference in the occurrence of NTD was highly significant ($\chi_1^2 = 6.21$; $P = 0.01$) between the multivitamin and trace element groups.

Another objective of the trial was to study the preventive effect of periconceptional folic acid-containing multivitamin supplementation on other CAs. The rate of three other CA groups showed a difference between the multivitamin and trace element groups.

Nine cases with CAs of the urinary tract were found in the trace element group and only two in the multivitamin group [$\chi_1^2 = 4.70$; $P = 0.03$; relative risk: 0.22 (0.05, 0.99)]. The difference was most obvious in the obstructive defects of the urinary system. One boy had urethral atresia with bilateral hydronephrosis (diagnosed prenatally and terminated) in the multivitamin group. Six (or five) cases had obstructive defects in the trace element group: two cases had bilateral hydronephrosis due to urethral obstruction sequence diagnosed prenatally (pregnancies were terminated) and three cases had unilateral pyelectasia and/or hydronephrosis caused by stenosis of the left ureteropelvic junction or ureter and had surgery. The sixth case had a similar clinical diagnosis but later it appeared that the obstruction was caused by discontinuity of the ureter due to a dystopic kidney. In addition, there were two offspring with renal agenesis (a fetus with bilateral agenesis was diagnosed prenatally, and in the second patient unilateral agenesis with ectopic kidney on the other side was detected after birth) in the trace element group. Finally, one girl had a left cystic kidney type II in the multivitamin group, while a boy had the exstrophy of the bladder sequence in the trace element group.

There were 10 infants with cardiovascular CAs in the multivitamin group and 20 in the trace element group ($\chi_1^2 = 3.69$; $P = 0.055$), relative risk being 0.48 (0.23, 1.03). The presence of cardiovascular CAs was confirmed before age 1 year by cardiological consultation (including echocardiography or cardiac catheterization), surgery, or autopsy. One case was familial (mentioned previously) in the multivitamin group, after the exclusion of this case, the difference was significant ($\chi_1^2 = 4.57$; $P = 0.032$). The difference of cardiovascular CAs is mainly explained by two cases of ventricular septal defect in the multivitamin group and eight cases in the trace element group ($\chi_1^2 = 3.81$; $P = 0.051$). These findings would not appear to be explicable by ascertainment biases in this study population. Some ventricular septal defects may have conotruncal septation anomalies. The combination of ventricular septal defects and conotruncal defects (in the multivitamin group: double outlet right ventricle 1, in the trace element group: tetralogy of Fallot 1, complete transposition 1) showed a significant difference between the multivitamin and trace element groups [$\chi_1^2 = 4.01$; $P = 0.045$; relative risk: 0.29 (0.08, 1.05)].

The number of clinical diagnoses of congenital hypertrophic pyloric stenosis (which is not a typical defect) also indicated some difference (2 vs. 8) between the study groups ($\chi_1^2 = 3.81$; $P = 0.051$). However, one case had no surgery in the trace element group, and according to our diagnostic criteria it was necessary to exclude this case from this analysis, resulting in an insignificant value ($\chi_1^2 = 2.95$; $P = 0.086$).

Among other CAs, two are worth mentioning here. There was no difference in the rate of cleft lip ± cleft palate in the two groups. Five cases with reduction CAs of the limbs were found in the trace element group and only one in the multivitamin group ($\chi_1^2 = 2.80$; $P = 0.094$).

DISCUSSION

The final evaluation of major CAs in the Hungarian trial resulted in four main findings. First, periconceptional multivitamin supplementation including 0.8 mg of folic acid can reduce significantly the first occurrence of NTD [Czeizel and Dudás, 1992; Czeizel et al., 1994]. Second, our findings do not agree with Tolarova [1982], who reported a protective effect of periconceptional multivitamin supplementation for cleft lip ± cleft palate. However, she used folic acid in a dose of 10 mg/day and our data lack the statistical power to exclude a protective effect for cleft lip ± cleft palate. Third, the total rate of offspring with major CAs in the multivitamin group was about half of that in the trace element group. After the exclusion of cases with NTD the difference of the total CA rates was 17.42/1,000 between the two study groups which is 6.3 times higher than the birth prevalence of NTD (2.78/1,000) in Hungary [Czeizel and Révész, 1970]. Fourth, periconceptional supplementation with a folic acid-containing multivitamin also seems to reduce the occurrence of CAs of the urinary tract (mainly obstructive defects) and cardiovascular CAs (mainly ventricular septal defects).

There are four arguments in favor of the validity of this analysis showing periconceptional multivitamin prevention for other major CAs in addition to NTDs. i) These findings were found in a randomised double-blind trial in which the ascertainment and later the personal examination of informative infants were made blindly, the dropout of pregnancies was very low, standardized diagnoses were used, all defects diagnosed prenatally and at birth were medically documented, and in the great majority of CAs the diagnosis was confirmed by personal examination later. ii) CAs of the urinary tract [Monie et al., 1954, 1957] and cardiovascular CAs [Baird et al., 1954; Monie and Nelson, 1963] were produced by folic acid deficiency in laboratory animal experiments. iii) There are also suggestive human data. In 1964 Hibbard reported a higher rate of CAs (3%) in the offspring of folate-deficient mothers than in controls (1.6%). Later Hibbard and Smithells [1965] showed a relationship between human embryopathies and a deficiency of folic acid metabolism based on the FIGLU (formiminoglutamic acid excretion) test. This test was positive in 62% of mothers with malformed infants and in 15% of mothers with normal infants. This difference was significant. iv) Folic acid acts as a cofactor for enzymes involved in DNA and RNA biosyn-

thesis and is also involved in the supply of methyl groups to the so-called methylation cycle which converts homocysteine to methionine [Scott et al., 1994]. In addition, folic acid and vitamin B_{12}, vitamin B_6, vitamin C, and zinc interact with one another in many metabolic pathways [Czeizel, 1995]. Thus, the decreased rate of CAs may also be related to overall vitamin intake. Cell division is exceptionally rapid at the critical stages of specific developmental fields in embryos. The cell's ability to increase the synthesis of nucleic acids and to methylate important compounds such as proteins, lipids, and DNA could be compromised by deficiency of folic acid and other vitamins resulting in impaired cell function. It may explain the multiendpoints of periconceptional multivitamin supplementation. In addition, genetically determined vitamin-dependency rather than vitamin-deficiency may have a causal role in the origin of NTDs and some other CAs.

However, in the evaluation of these findings, three factors should be considered: i) Besides NTD, other CA groups were not the outcomes related to the main hypothesis that the trial was designed to test. There was no specific hypothesis that a folic acid-containing multivitamin would prevent other major CAs because they are all unlikely to share the same etiology. Furthermore, given that many comparisons were made, "significant" associations are likely to be observed by chance alone. Thus, this report should be regarded as a hypothesis-generating study encouraging others to see if the results can be repeated. ii) The observed rate of ventricular septal defects and obstructive urinary tract defects in the trace element group is somewhat but not significantly higher than expected based on the previous Hungarian figures. This may be connected with more sensitive diagnostic procedures (ultrasound, echocardiography, etc.). iii) Finally, CAs of the urinary tract and cardiovascular system are heterogeneous, thus, the different types within these CA groups may have different causes. However, this is the case in NTDs as well.

ACKNOWLEDGMENTS

I am grateful to Dr. L.B. Holmes (Boston) for the help in the classification of defects; to Dr. A. Bendich (Nutley), Dr. J.D. Erickson (Atlanta), Dr. K. Méhes (Pécs), and Dr. R.W. Smithells (Leeds) for a review of the manuscript; to Dr. A. Hanck, G. Kunovits and Dr. J. Grueter of Hoffmann-La Roche for supplying the supplements and to Dr. Marta Dobo, Dr. I. Dudas, Dr. Zs. Kalmár, Dr. J. Métneki, and E. Halenkar, A. Horvath, K. Makai, M. Zalatnay for their assistance. After submitting this paper, a case-control study was published (Li D.K., Daling T.R., et al.: Epidemiology 6:212–218, 1995), which found a protective effect of periconceptional folic acid-containing multivitamin supplementation for fetal urinary tract defects.

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